

Appl. No. 09/402,488
Amdt. Dated January 28, 2004
Reply to Office action of September 17, 2003

Amendments to th Claim :

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (Previously amended): A method for the preparation of a recombinant polypeptide comprising

- a) transforming a host cell with an expression vector comprising:
 - (1) a nucleic acid sequence capable of regulating transcription in a host cell, operatively linked to
 - (2) a chimeric nucleic acid sequence encoding a fusion protein, the chimeric nucleic acid sequence comprising (a) a nucleic acid sequence encoding a chymosin pro-peptide, linked in reading frame to (b) a nucleic acid sequence heterologous to the pro-peptide and encoding the recombinant polypeptide, wherein the heterologous nucleic acid sequence is located immediately downstream of the nucleic acid sequence encoding the chymosin pro-peptide; operatively linked to
 - (3) a nucleic acid sequence encoding a termination region functional in said host cell,
- b) growing the host cell to produce said fusion protein; and
- c) adding a mature form of an autocatalytically maturing aspartic protease, that is capable of cleaving the chymosin pro-peptide, to the fusion protein so that the chymosin pro-peptide is cleaved from the fusion protein to release the recombinant polypeptide.

Claims 2-3 (Canceled).

Claim 4 (Previously amended): The method according to claim 1 wherein said aspartic protease added in step (c) is selected from the group consisting of chymosin, pepsin, HIV-1 protease, pepsinogen, cathepsin and yeast proteinase A.

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Claim 5 (Previously amended): The method according to claim 1 wherein the recombinant polypeptide is hirudin or carp growth hormone.

Claim 6 (Previously amended): The method according to claim 1 wherein the chimeric nucleic acid sequence does not include a sequence encoding a mature form of chymosin.

Claim 7 (Previously amended): The method according to claim 1 wherein the pH is from about 2 to about 7 in step (c).

Claim 8 (Currently amended): The A method according to claim 7 wherein the pH is from about 2 to about 4.5.

Claim 9 (Previously amended): The method according to claim 1 wherein step (c) takes place under in vitro conditions.

Claim 10 (Previously amended): The method according to claim 1 wherein step (c) takes place under in vivo conditions.

Claim 11 (Canceled).

Claim 12 (Currently amended): The method according to claim 10 wherein the in vivo conditions are those prevalent in a tissue or bodily fluid of an animal and wherein the tissue or bodily fluid comprises the milk, the stomach, or the gut or the of said animal.

Claim 13 (Previously amended): The method according to claim 1 wherein the mature form of the aspartic protease added in step (c) is chymosin.

Claim 14 (Previously amended): The method according to claim 1 wherein the aspartic protease added in step (c) is heterologous to the chymosin pro-peptide.

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Claim 15 (Previously amended): The method according to claim 13 wherein the chymosin is added under in vitro conditions.

Claim 16 (Previously amended): The method according to claim 13 wherein the chymosin is added under in vivo conditions.

Claim 17 (Canceled).

Claim 18 (Previously amended): The method according to claim 16 wherein said in vivo conditions take place in a tissue or bodily fluid of an animal and wherein the tissue or bodily fluid is a stomach, gut, or milk of said animal.

Claim 19 (Previously amended): The method according to claim 1 wherein said nucleic acid sequences are deoxyribonucleic acid (DNA) sequences.

Claim 20 (Currently amended): A chimeric nucleic acid sequence encoding a fusion protein comprising (a) a nucleic acid sequence encoding a full length chymosin pro-peptide and (b) a nucleic acid sequence encoding a polypeptide that is heterologous to the chymosin pro-peptide, wherein the heterologous nucleic acid sequence is located immediately downstream of the nucleic acid sequence encoding the chymosin pro-peptide.

Claims 21-23 (Canceled).

Claim 24 (Previously amended): The chimeric nucleic acid sequence according to claim 20 wherein the polypeptide is hirudin or carp growth hormone.

Claim 25 (Previously amended): The chimeric nucleic acid sequence according to claim 20 which does not include a sequence encoding a mature form of chymosin.

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Claim 26 (Previously amended): The chimeric nucleic acid sequence according to claim 20 wherein said nucleic acid sequences are deoxyribonucleic acid (DNA) sequences.

Claim 27 (Previously amended): The chimeric nucleic acid sequence according to claim 26 wherein the chimeric sequence is as shown in SEQ ID NO:1 or SEQ ID NO:3.

Claim 28 (Previously amended): An expression vector comprising the chimeric nucleic acid sequence according to claim 20 and a regulatory sequence suitable for expression in a host cell.

Claim 29 (Original): A transformed host cell containing an expression vector according to claim 28.

Claim 30 (Original): A transformed host cell containing an expression vector according to claim 28 wherein the host cell is a bacterial cell, a fungal cell, a plant cell or an animal cell.

Claims 31-40 (Canceled).

Claim 41 (Currently amended): A composition comprising a chimeric nucleic acid sequence encoding a fusion protein, the chimeric nucleic acid sequence comprising (a) a first nucleic acid sequence encoding a full length chymosin pro-peptide and (b) a second nucleic acid sequence encoding a polypeptide that is heterologous to the chymosin pro-peptide, wherein the heterologous nucleic acid sequence is located immediately downstream of the nucleic acid sequence encoding the chymosin pro-peptide.

Claim 42 (Canceled).

Claim 43 (Previously amended): The composition according to claim 41 wherein the nucleic acid sequences are deoxyribonucleic acid (DNA) sequences.

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Claim 44 (Previously amended): The composition according to claim 41 wherein said chimeric nucleic acid sequence does not include a sequence encoding a mature form of chymosin.

Claim 45-47 (Canceled).